Remarks

In accordance with the provisions of 37 C.F.R. §§ 1.821-1.825, Applicants have amended the specification to correct the misnumbering of certain SEQ ID NOS in the specification and to insert an amended written copy of the Substitute Sequence Listing. A copy of the Substitute Sequence Listing in computer readable form, the contents of which are identical to the written form of the Substitute Sequence Listing, is also enclosed.

This Amendment does not introduce any new subject matter as support is found in the application as filed.

Applicants kindly remind the Patent and Trademark Office that the correspondence address has been changed to that listed below the signature line, and the attorney docket number has been changed to 36119-125(US10).

Conclusions

No additional fees are believed to be due in connection with this filing. However, please charge any underpayments or credit any overpayments to our Deposit Account No. 08-0219.

If there are any questions, please contact the undersigned at the telephone number indicated below.

Respectfully submitted,
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Date: July 11, 2002

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Marked-Up Version of Amended Paragraphs

On page 18, line 33 through page 19, line 33, please delete the paragraph and replace it with the following paragraph:

Accordingly, to expand a population of CD8+ T cells, an antibody, such as monoclonal antibody ES5.2D8, or other antibody which recognizes the same 27 kD ligand as monoclonal antibody ES5.2D8 can be used. As described in Example 10, the epitope recognized by the monoclonal antibody ES5.2D8 was identified by screening a phage display library (PDL). Antibodies which bind to the same epitope as the monoclonal antibody ES5.2D8 are within the scope of the invention. Such antibodies can be produced by immunization with a peptide fragment including the epitope or with the native 27 kD antigen. The term "epitope," as used herein, refers to the actual structural portion of the antigen that is immunologically bound by an antibody combining site. The term is also used interchangeably with "antigenic determinant". A preferred epitope which is bound by an antibody or other ligand which is to be used to stimulate a CD8+ T cell population includes or encompasses, an amino acid sequence:

$(Xaa_1)_n$-Gly-Xaa_2-Trp-Leu-Xaa_3-Xaa_4-Asp(Glu)-$(Xaa_5)_n$ (SEQ ID NO: [9] 5),

wherein Xaa_4 may or may not be present, Xaa_1, Xaa_2, Xaa_3, Xaa_4 and Xaa_5 are any amino acid residue and n = 0-20, more preferably 0-10, even more preferably 0-5, and most preferably 0-3. In a preferred embodiment, Xaa_2 is Cys, Ile or Leu, Xaa_3 is Leu or Arg and Xaa_4, if present, is Arg, Pro or Phe. As described in Example 10, the monoclonal antibody ES5.2D8, which specifically binds a 27 kD antigen on activated T cells was used to screen a cDNA library from activated T cells to isolate a clone encoding the antigen. Amino acid sequence analysis identified the antigen as CD9 (SEQ ID NO: [10] 6). In the native human CD9 molecule, epitope defined by phage display library screening is located at amino acid residues 31-37 (i.e., G L W L R F D (SEQ ID NO: [13] 7)). Accordingly, Xaa_1 and Xaa_4 are typically additional amino acid residues found at either the amino or carboxy side, or both the amino and carboxy sides, of the core epitope in the human CD9 (the full-length amino acid sequence of which is shown in SEQ ID NO: [10] 6). It will be appreciated by those skilled in the art that in the native protein, additional non-contiguous amino acid residues may also contribute to the conformational epitope recognized by the antibody. Synthetic peptides encompassing the epitope can be created which includes other amino acid residues flanking the core six amino acid residues (i.e. Xaa can alternatively be other amino acid residues than those found in the native CD9 protein). These flanking amino acid residues can function to alter the properties of the resulting peptide, for example to increase the solubility, enhance the

immunogenicity or promote dimerization of the resultant peptide. When the peptide is to be used as an immunogen, one or more charged amino acids (e.g. lysine, arginine) can be included to increase the solubility of the peptide and/or enhance the immunogenicity of the peptide. Alternatively, cysteine residues can be included to increase the dimerization of the resulting peptide.

On page 27, line 13 through page 28, line 8, please delete the paragraph and replace it with the following paragraph:

Alternative to a CD28-expressing cell or an isolated CD28 protein, peptide fragments of CD28 or other surface antigen such as CD9 can be used as immunogens to generate antibodies. For example, the CD9 epitope bound by the ES5.2D8 monoclonal antibody comprises an amino acid sequence: $(Xaa_1)_n$-Gly-Xaa_2-Trp-Leu-Xaa_3-Xaa_4-Asp(Glu)-$(Xaa_5)_n$ (SEQ ID NO: [9] 5), wherein Xaa_4 may or may not be present, Xaa_1, Xaa_2, Xaa_3, Xaa_4 and Xaa_5 are any amino acid residue and n = 0-20, more preferably 0-10, even more preferably 0-5, and most preferably 0-3. In a preferred embodiment, Xaa_2 is Cys, Ile or Leu, Xaa_3 is Leu or Arg and Xaa_4, if present, is Arg, Pro or Phe. Thus, a peptide having the amino acid sequence of SEQ ID NO: 5 can be used as an immunogen. Accordingly, the invention further encompasses an isolated CD9 peptide comprising an amino acid sequence: $(Xaa_1)_n$-Gly-Xaa_2-Trp-Leu-Xaa_3-Xaa_4-Asp(Glu)-$(Xaa_5)_n$ (SEQ ID NO: [9] 5), wherein Xaa_4 may or may not be present, Xaa_1, Xaa_2, Xaa_3, Xaa_4 and Xaa_5 are any amino acid residue and n = 0-20, more preferably 0-10, even more preferably 0-5, and most preferably 0-3. In a preferred embodiment, Xaa_2 is Cys, Ile or Leu, Xaa_3 is Leu or Arg and Xaa_4, if present, is Arg, Pro or Phe. Alternatively, it has been found that the ES5.2D8 monoclonal antibody cross-reacts with a number of other peptide sequences (determined by phage display technology as described in Example 3). Examples of these other peptide sequences are shown below:

2D8#2 (SEQ ID NO: [5] 8)	H Q F C D H W **G C W L L R** E T H I F T P
2D8#4 (SEQ ID NO: [6] 8)	H Q F C D H W **G C W L L R** E T H I F T P
2D8#10 (SEQ ID NO: [7] 8)	H Q F C D H W **G C W L L R** E T H I F T P
2D8#6 (SEQ ID NO: [8] 9)	L R L V L E D P **G I W L R P D** Y F F P A
phage 2D8#2, 4, 10 (SEQ ID NO: [11] 10)	G C W L L R E
phage 2D8#6 (SEQ ID NO: [12] 11)	G I W L R P D
CD9 sequence (SEQ ID NO: [13] 7)	G L W L R F D

Any of these peptides, or other peptides containing a stretch of seven amino acids bracketed in bold type (representing the epitope bound by the antibody) possibly flanked by alternative amino acid residues, can also be used as immunogens to produce an antibody for use in the methods of the invention and are encompassed by the invention. For use as immunogens, peptides can be modified to increase solubility and/or enhance immunogenicity as described above.

On page 48, lines 10 through 18, please delete the paragraph and replace it with the following paragraph:

Sequence analysis of these clones demonstrated that three of the seven sequences were identical and a fourth was similar:

2D8#2 (SEQ ID NO: [5] 8)	H Q F C D H W **G C W L L R E** T H I F T P
2D8#4 (SEQ ID NO: [6] 8)	H Q F C D H W **G C W L L R E** T H I F T P
2D8#10 (SEQ ID NO: [7] 8)	H Q F C D H W **G C W L L R E** T H I F T P
2D8#6 (SEQ ID NO: [8] 9)	L R L V L E D P **G I W L R P D** Y F F P A

Based on this data an epitope of **G X W L X D/E** (SEQ ID NO: [9] 12) was proposed.

On page 49, lines 19 through 29, please delete the paragraph and replace it with the following paragraph:

The DNA from 4 μg of AMV reverse transcription and 2.0 μg of Moloney MLV reverse transcription were combined. Non-selfcomplementary BstXI adaptors were added to the DNA as follows: The double-stranded cDNA from 6 μg of poly(A)+RNA was incubated with 3.6 μg of a kinased oligonucleotide of the sequence CTTTAGAGCACA (SEQ ID NO: [9] 13) and 2.4 μg of a kinased oligonucleotide of the sequence CTCTAAAG (SEQ ID NO: 14) in a solution containing 6 mM Tris, pH 7.5, 6 mM $MgCl_2$, 5mM NaCl, 350 μg/ml bovine serum albumin, 7 mM mercaptoethanol, 0.1 mM ATP, 2 mM dithiothreitol, 1 mM spermidine, and 600 units T4 DNA ligase in a total volume of 0.45 ml at 15 °C for 16 hours. EDTA was added to 34 mM and the solution was extracted with an equal volume of 50 %

phenol, 49 % chloroform, 1 % isoamyl alcohol. DNA was precipitated with two volumes of ethanol in the presence of 2.5 M ammonium acetate.

On page 50, line 35 through page 51, line2, please delete the paragraph and replace it with the following paragraph:

BESTFIT analysis of the phage epitopes of mAb 2D8 to the amino acid sequence of CD9 revealed a close match:

Phage 2D8#2, 4, 10 (SEQ ID NO: [11] 10): G C W L L R E
Phage 2D8#6 (SEQ ID NO: [12] 11): G I W L R P D
CD9 sequence (SEQ ID NO: [13] 7): G L W L R F D

On page 51, lines 15 through 24, please delete the paragraph and replace it with the following paragraph:

Cd9_Human Length: 227 May 25, 1994 14:10 Type: P Check: 1577

(SEQ ID NO: [10] 6)

1 PVKGGTKCIK YLLFGFNFIF WLAGIAVLAI GLWLRFDSQT KSIFEQETNN
51 NNSSFYTGVY ILIGAGALMM LVGFLGCCGA VQESQCMLGL FFGFLLVIFA
101 IEIAAAIWGY SHKDEVIKEV QEFYKDTYNK LKTKDEPQRE TLKAIHYALN
151 CCGLAGGVEQ FISDICPKKD VLETFTVKSC PDAIKEVFDN KFHIIGAVGI
201 GIAVVMIFGM IFSMILCCAI RRNREMV